

Appl. No. 09/679,043  
Amendment dated: September 16, 2003  
Appeal Brief Due: September 19, 2003

### **REMARKS**

Applicants acknowledge with appreciation the courtesy of the interview extended the undersigned attorney by Examiner Cook on August 12, 2003. As reflected in the Interview Summary Record, claim 1 was discussed as well as the amendment to the claims and it is believed that claim 1, which has now been rewritten as claim 28 is in full compliance with 35 U.S.C. 112 and is clearly patentable over the references of record subject to the Examiner's further detailed consideration and review of additional prior art. An RCE is filed herewith to allow for additional searching by the Examiner. If free of the prior art, Examiner indicates that the application will be allowed.

Claims 1-8, and 13-25 have been cancelled from the application and new claims 28-49 have been added. Claim 28 corresponds to claim 1 and includes the amendments discussed at the interview in order to overcome the rejection under 35 U.S.C. 112. As recognized at the interview, the amendment previously made of claim 1 included a typographical error and the determination, as recognized by the Examiner is for transcobalamin II bound cobalamin (holo-TCII).

In addition, all other dependent claims have been rewritten in appropriate form and made either directly or indirectly dependent upon claim 28. Claim 35 corresponds to claim 8, claims 40, 41 and 42 correspond to claims 17, 18 and 19.

In view of the understanding that claim 28 would be allowable if free from the prior art, it is believed that there is a generic claim in the application and all of the claims now present are directed to the elected invention. Consideration of all the claims on the merits is now in order and is most respectfully requested.

The rejection of claim 1 under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention has been carefully considered. However, in view of the replacement of claim 1 with claim 28 and the following comments, Applicants believe that this rejection has been obviated.

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The Examiner states in the Official Action in item 7 that newly amended claim 1 is directed to the determination of "transcobalamin II cobalamin", however no such compound is identified in the disclosure and Applicant is invited to show support for the term in the instant application.

The term "Transcobalamin II cobalamin" is essentially meaningless and should, of course, have been "Transcobalamin II bound cobalamin", as in the original claim set and as now used in claim 28 and as discussed at the interview. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claim 1 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which application regards as the invention has been carefully considered.

The Examiner states that claim 1 is vague and indefinite because it is not clear what the assay method will detect. Initially the claim is directed to the determination of "transcobalamin II cobalamin". However, the term is not found in the disclosure. The claim further recites the measurement of TC II or cobalamin but the claim utilizes TC II or holo-TC II to separate bound fraction from unbound fraction. Several terms appear to be used interchangeably in the claim which makes the method unclear.

Applicants have amended the claims with respect to the term objected to in this section of the Office Action which no longer appears in the claims and consistent language is used throughout. Accordingly, this aspect of the rejection should be withdrawn.

The Examiner also objects to the lack of an explicit separation or removal step in the main claim. As indicated above, the actual removal of the apo-species is not essential to the functioning of the method and was specifically indicated as unnecessary in the text. It is, however, essential to the functioning of the method that the apo-form of TC no longer participates in the assay. As discussed at the interview, and in the previous amendments in this application, there are four protein species that are of significance to the present invention, these being holo-TCII, holo-HC, apo-TCII and apo-HC. The holo proteins contain bound cobalamin (cobalamin = vitamin B12) and the

*maintain*

*\*invention*

apo proteins do not. The TCII components contain the TCII protein while the HC components contain at least one of the haptocorrin proteins. The species which this invention provides a method for measuring is "holo-TCII", or in other words the amount of cobalamin that is bound to the TCII protein. Holo-TCII is the smaller of the two cobalamin containing fractions since at least 4 times as much of the cobalamin is present in the form of holo-HC. Almost no free (unbound) cobalamin exists in body fluid samples.

NOT IN CLAIMS?  
The central feature of the present invention is that it involves the step of pre-binding the apo-species. The purpose of this step is to remove the apo-species from the subsequent analysis. This may be by physically removing the apo-HC and apo-TC from the sample, or equally simply by masking the apo-forms so that they no longer participate in the later steps. This could be, for example, by binding the apo-forms to a substrate so that they are no longer in solution (whether or not the substrate was physically removed from the sample) or could be by binding to the cobalamin binding site in the apo proteins in a way that masks another binding site such that the subsequent assay does not recognise this latter site. In none of the cited art is there any indication that this pre-distinguishing step was considered or suggested.

The specification makes it clear that the inventors did not intend that the physical removal of the apo-species be essential but simply that the pre-binding step remove the apo-forms from participation in the subsequent assay. The last paragraph of page 16 states:

NOT IN CLAIMS  
"In a preferred embodiment, in the preliminary separation step, the binding of apo TC II and apo HC to cobalamin, analogues or fragments thereof takes place at a site or in such a manner which inhibits subsequent recognition and binding of the immobilised cobalamin bound TC II by the non-immobilised ligand or binding partner for TC II. In this embodiment there is no need to isolate the holo-TC II and holo-HC from the bound apo forms before performing the assay of the invention. In this embodiment, the site against which the non-immobilised binding partner or ligand is directed is very important and should be an epitope on TC II which becomes masked or shielded or otherwise

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unavailable for binding when the apo TC-II and apo-HC becomes immobilised on the cobalamin, analogue or fragment thereof."

NOT IN CLAIM  
In view of the above, a number of amendments have been made to the enclosed claim set. In particular, claim 28 has been added and clearly defines the invention. See for example lines 25-29 of the specification at page 15. In addition, new independent claim 49 has been introduced stating that the apo-form is removed. This is clearly one important embodiment of the invention and an example of how the apo-TCII may be made unavailable.

If the bound apo forms are removed from the sample then clearly they will not affect the holo-TCII measurement. However they need not affect that measurement if they are not removed as long as the measurement step is selected appropriately. Thus for example if a labelled holo-TCII specific ligand is used there could be no "noise" from the bound apo forms. Likewise if one uses a labelled ligand which recognizes both apo and holoTCII when in solution but does not recognize the immobilized apo-TCII, then the measurement is uncontaminated. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claim 1 under 35 U.S.C. 103(a) as being unpatentable over Morelli et al. in view of Maggio has been carefully considered but is most respectfully traversed in view of the following comments.

The Examiner states that Morelli teaches a method for determining "transcobalamin II (TCII) bound cobalamin or vitamin B12 (TCII-B12)". In fact, Morelli only relates to a method for determining the total amount of TCII (protein) in the sample, with no distinction drawn between the apo- and holo-forms. This is made very clear in the introduction/abstract on page 645 where it is stated that "The assay was responsive to either TCII or TCII-B12". Since the assay responds equally to apo-TCII and holo-TCII, there can be no discrimination between these. The first paragraph of the main text also makes it quite clear that it is total TCII and not holo-TCII which is to be assayed. These points were reiterated at the interview.

It is believe that at the interview the sections in the Official Action, such as where the Examiner states "B12 was bound to TCII and then subsequently contacted with a specific binding ligand for the complex (TCII-B12). Free and bound fractions were collected and utilized to determine the amount of (TCII-B12) present in the sample" were clarified to ensure that the Examiner understands what is being described and claimed in the present application and the prior art.

In fact, Morelli describes two separate assays for total TCII. The first involves saturating the TCII in a sample with cobalamin (B12) to convert the apo-TCII to holo-TCII, and then measuring the cobalamin released when all the TCII (now all converted to holo-TCII) is denatured. This is the assay described as "Measurement of total TCII-B12 independently of immunoassay" on page 646. The assay measures the cobalamin released from holo-TCII but only once all the TCII has been converted to holo-form. The assay is thus for total TCII by means of converting it to holo-TCII. It tells one of ordinary skill in the art nothing about the amount of holo-TCII in the original sample. To quote from page 646 lines 45-46 "Since the TCII was saturated [with B12] and the TCII-B12 isolated, the measure of B12 was also an indirect measure of [total] TC". No specific binding ligand is involved with this method, the TCII protein is simply precipitated with ammonium sulphate, leaving the B12 in solution.

The section on pages 648-9 is a completely different assay from the one considered on page 646. This uses the ratio of "bound" TCII to "unbound". This is the ratio of TCII associated with a TCII binding antibody to that TCII that is free in solution. The ligand is specific for TCII and not for holo-TCII (see paragraph bridging pages 649-650 and especially Fig. 2). Since the number of binding sites is fixed, if the amount of TCII in the sample increases then a lower proportion will bind to the ligand. The TCII is first labelled with radioactive cobalamin to allow detection and then partitioned between the binder and the solution. It makes no difference to the ratio whether the original sample contains holo-TCII or apo-TCII, the measurement will yield the same ratio providing there is enough radiolabel to give a detectable signal on the ligand and

in solution. It is exactly equivalent to saying that if half the people on a bus are standing and there are 20 seats then there must be 40 people in total.

The two methods described above are two independent ways of assessing the total TCII content of a sample. Applicants most respectfully submit that the passage quoted clearly indicates that the Examiner is mixing the two up and not appreciating the aim of the paper. The fact that these are different and completely separate methods is best illustrated by Figure 4, which compares the two.

Morelli differs fundamentally from the present invention in that:

- a) It is an assay for total TCII and not holo-TCII (which is a very small fraction of total TCII).
- b) There is no pre-binding step in Morelli to remove/inactivate the apo-species.
- c) It does not achieve b) by binding to a cobalamin or analogue thereof.

The Examiner asserts that the combination of Maggi with Morelli would lead the skilled worker to an assay of the present invention. This, however, cannot be the case because neither reference teaches anything about integers a-c above.

The Examiner rejects Applicants' previous argument on the grounds that this point is not reflected in the claim. This is, however, because the essential feature of the apo-binding step has not been fully appreciated. Once the apo-components are rendered inactive in the remainder of the assay, it does not matter that TCII is measured rather than holo-TCII because the only active TCII remaining is that portion originally present as holo-TCII. This was illustrated in the figure submitted with the last response, in which it can be seen that the TC remaining is that bound to cobalamin. This figure was reviewed at the interview. An identical result is achieved if the apo-TC is simply "masked" by the pre-binding rather than removed.

Neither Allen nor Herbert provide an assay method for holo-TCII comprising pre-binding to remove or inactivate the apo-species, which is the key feature of the present claims. Therefore neither teach the present invention, either individually or in

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any combination. Accordingly, it is most respectfully requested that this rejection be withdrawn.

It is urged in the Official Action that an application in which the benefits of an earlier application are desired must contain a specific reference to the prior applications in the first sentence of the specification as required in 37 CFR 1.78. However, Applicants have complied with this rule and did list the prior application since this application is a continuation of international application PCT/GB99/03127, filed September 20, 1999. The application referred to in the Official Action is a priority document and is listed in the 119 box of the executed Declaration. Accordingly, this objection should be withdrawn. The Examiner agreed at the interview to look into this point.

In view of the above comments and further amendments to the claims, favorable reconsideration and allowance of all of the claims now present in the application are most respectfully requested.

Respectfully submitted,

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